



# UNDERSTANDING THE EFFECT OF DEFORMED WING VIRUS AND IMIDACLOPRID INSECTICIDE ON COLONY COLLAPSE DISORDER

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## ABSTRACT

It is essential to recognize that bees and other pollinators play a crucial role in ecosystems. Indirectly or directly, all human food sources rely on bees for survival. However, in recent years, there has been a concerning decline in honeybee colonies due to various factors such as pesticides, diseases, and poor nutrition. This phenomenon is referred to as Colony Collapse Disorder (CCD). The effects of exposure to the imidacloprid insecticide and DWV infection on the *Apis mellifera* brain were examined in this study using publicly available RNA-seq datasets.

## INTRODUCTION

Bees are a vital component of biodiversity as they serve as essential pollinators. Their importance lies in the fact that they are responsible for pollinating around 85% of all human food crops. However, honeybee colonies have been on the decline in recent years due to several factors, including pesticides, diseases, and poor nutrition. This phenomenon, known as Colony Collapse Disorder (CCD), was first reported in the United States in 2006.

CCD is characterized by the disappearance of a significant portion of a colony's worker bees, leaving behind only the queen and a few young bees. Various stress factors, acting either independently or in combination, are thought to contribute to the vulnerability of bees, allowing pathogens such as Deformed Wing Virus (DWV) to contaminate and destroy colonies.

Among honeybee colonies, the Varroa destructor is considered the most significant mite globally due to its ability to attack different growth phases of *A. mellifera*. The mite's negative impact is derived from its role as a viral reservoir and transmitter of honeybee-associated viruses, such as DWV. Infection with DWV, a common virus affecting honeybees, can have various negative effects on honeybee health and can lead to CCD.

Imidacloprid, an insecticide that mimics nicotine, is a common insecticide known to have negative effects on honeybee health. In 2022, the ban on imidacloprid insecticide was extended due to expanding evidence that it can harm not only domesticated honeybees but also pollinators.

The objective of this study is to analyze the gene expression of bees infected with DWV and bees exposed to imidacloprid using Gene Ontology (GO). GO enrichment analysis is a method used to identify the over-representation of a particular set of genes or gene products in a functional category.

## Materials & Methods

### RNA-Seq Analysis

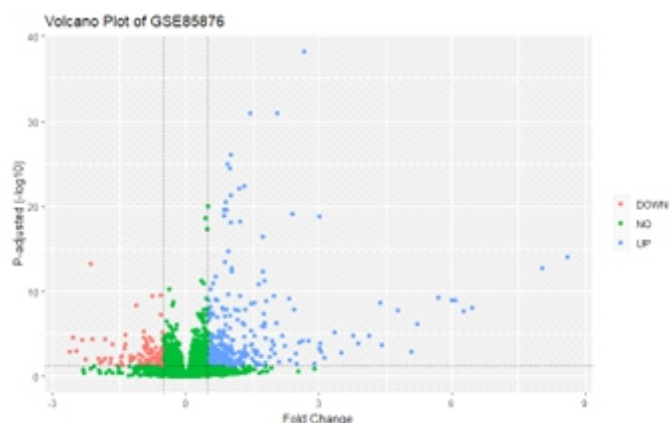
In order to find out the differentially expressed genes on the brain of *A. mellifera* during the exposure to the imidacloprid and DWV exposure, RNA-seq analysis was performed. Publicly available datasets were used for this purpose. For the differential gene expression (DGE) analysis, DESeq2 R package was used and a standard DESeq2 workflow was performed.

### Overrepresentation Analysis

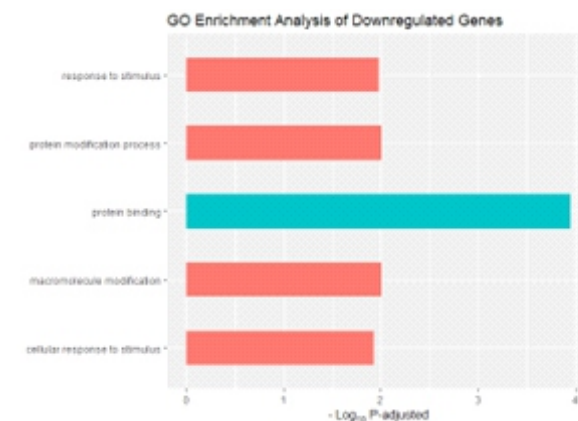
In order to understand the enrichment of differentially expressed genes, overrepresentation analysis was performed by using the gprofiler2 R package. Visualizations of the results were performed by using the ggplot2 R package.

## Results

Differential genes analysis using publicly available data (NCBI ACCESSION: GSE85876) was used. I found that 404 genes are significantly differentially expressed (absolute logFC  $\geq 0.5$  and p-adjusted value  $\leq 0.05$ ) among those 404 genes 284 of them were found to be upregulated in the DWV-infected group and 120 of them were found to be downregulated.



This graph shows that the different gene expressions in the DWV tested honeybees.



The transcriptomic effects of DWV infection on *Apis mellifera* were examined in this study, with a focus on downregulated genes and their corresponding functional categories.

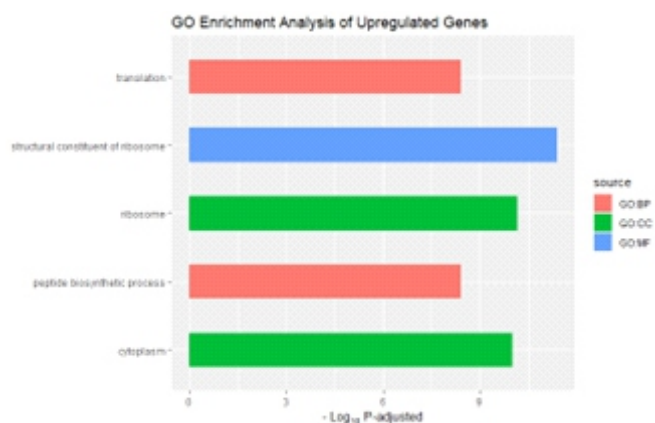
The data indicate that DWV infection leads to a reduction in the cell activity of genes that respond to external stimuli, such as stress, pathogens, and environmental changes.

Moreover, protein modification genes that are involved in post-translational modifications such as phosphorylation, acetylation, or glycosylation were also found to be downregulated. Protein binding activity, which is crucial for processes like signal transduction, transcriptional regulation, and transport, was also affected.

Additionally, the study revealed a reduction in macromolecule modification, indicating that DWV could potentially modify other macromolecules such as nucleic acids or polysaccharides, much like protein modification.

Finally, DWV was found to have a negative impact on the cellular response to var-

ious types of stimuli. These findings highlight the significant impact of DWV infection on the gene expression of honeybees and shed light on the various mechanisms through which this virus affects honeybee health.



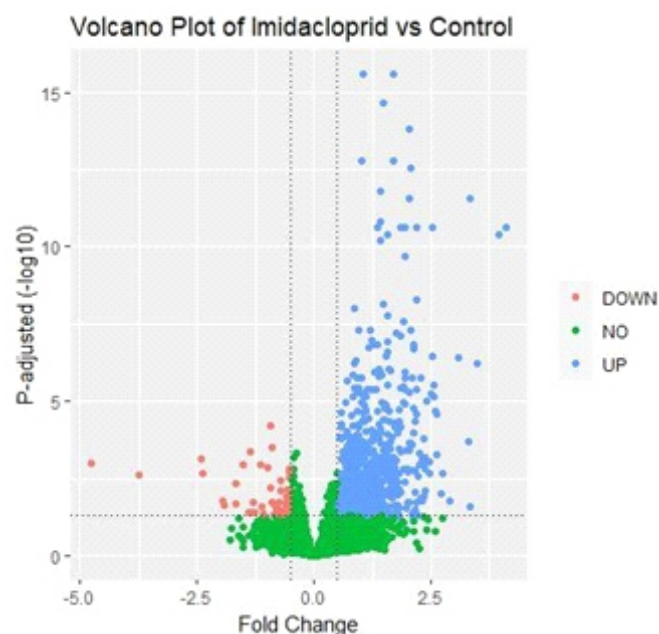
Upregulated genes in bees with DWV are enriched for Gene Ontology (GO) terms related to various cellular processes. These processes are likely to be impacted by the virus, and they include metabolic processes, cellular component organization, and signaling.

Based on the provided data, we can draw some preliminary conclusions about the upregulated genes in bees with DWV:

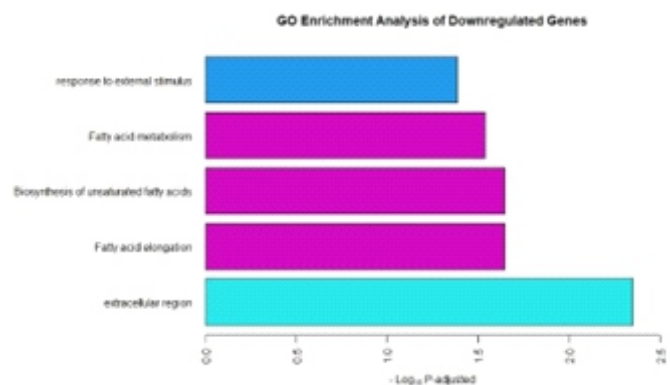
The ribosome-related genes are differentially expressed in the affected bees including a structural constituent of the ribosome and ribosome itself, these changes affect the translation process. It can also be seen from the data that translation process is enriched.

Peptide biosynthesis is also affected. Gene related to peptide biosynthesis is differentially expressed. This could indicate that the bees are having difficulty producing peptides, which could affect various cellular processes.

The cytoplasm is affected by DWV. Genes related to the cytoplasm are differentially expressed. This could indicate that the virus is impacting various cellular processes that occur in the cytoplasm.



This graph shows that the different gene expressions in the imidacloprid tested honeybees.



In this study, the downregulated genes affected by imidacloprid were analyzed using Gene Ontology (GO) enrichment analysis to identify the enriched functional categories. The data revealed that the following genes were downregulated by imidacloprid:

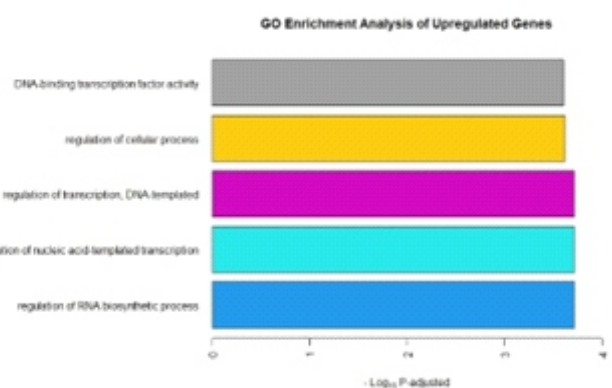
Firstly, response to stimuli was reduced in the imidacloprid-downregulated genes, which is a similar change observed in the bees affected by DWV. This change is significant due to the gene's role in responding to external stimuli such as stress, pathogens, or environmental changes.

Secondly, fatty acid metabolism was reduced in the infected bees, indicating that imidacloprid affects the regular activity of this pathway. Fatty acids are essential building blocks that affect energy production, signaling, and membrane structure.

Thirdly, unsaturated fatty acid biosynthesis was also reduced, indicating that imidacloprid insecticides impact this pathway. Unsaturated fatty acids are crucial for various cellular processes and required for cell membrane function.

Fourthly, fatty acid elongation was decreased in the cells affected by imidacloprid, which is necessary for complex lipid biosynthesis and the regulation of various cellular processes.

Lastly, imidacloprid was found to affect extracellular regions, which include structures such as the extracellular matrix and secreted proteins. The extracellular region is essential in cell signaling and communication.



In this study, upregulated genes affected by imidacloprid were analyzed.

Based on the data, the following upregulated genes were affected by imidacloprid:

DNA-binding transcription factor activity was found to be enriched in upregulated genes, indicating that these genes play a role in regulating gene expression through DNA binding. Transcription factors are important in various cellular processes such as development, differentiation, and response to environmental stimuli.

The regulation of cellular processes is also overrepresented, suggesting that the upregulated genes are involved in the regulation of multiple cellular processes, including signaling pathways such as the MAPK/ERK pathway, the PI3K/Akt pathway, and the JAK/STAT pathway.

DNA-templated transcription regulation is also enriched by imidacloprid, indicating that the insecticide may play a role in regulating DNA transcription.

Regulation of nucleic acid-templated transcription is enriched, suggesting that imidacloprid may be involved in the regulation of various types of nucleic acid-templated transcription, including RNA transcription.

Additionally, regulation of the RNA biosynthetic process is enriched by imidacloprid, which is required for gene expression and regulated by various transcription factors and co-factors.

### Discussion

The provided data suggests that DWV affects various cellular processes in bees, including the enrichment of translation, peptide biosynthesis, and cytoplasmic functions. This virus also decreases the response to stimuli, protein modification, protein binding, and macromolecule modification functions. These findings could help to further understand the mechanisms by which DWV infection affects honeybee health.

The genes affected by imidacloprid are involved in various cellular processes, including a decrease in response to stimuli, fatty acid metabolism, biosynthesis of unsaturated fatty acids, fatty acid elongation, and the extracellular region. The pesticide also affects the enrichment of DNA-binding transcription factor activity, regulation of transcription (DNA-templated and nucleic acid-templated), and regulation of the RNA biosynthetic process. These findings could help to further understand the mechanisms by which imidacloprid affects honeybee health.

While there are some similarities in the cellular processes affected by DWV and imidacloprid, it is important to note that they are two separate stressors that can have different impacts on honeybee health. DWV is a virus that can weaken bees and make them more susceptible to other stressors, while imidacloprid is a pesticide that can directly affect the physiological functions of bees. By understanding how these stressors affect honeybees at the cellular level, we can better understand the mechanisms of colony collapse disorder (CCD).

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